EXHIBIT A

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- one plate of HUVEC P7 was trypsinised and 2×10^5 cells were plated per insert (8µm, coated with fibronectin), 29 inserts total. The media was changed every day until they reached complete confluency.

Neutrophil migration assay

Neutrophils migrate in response to a gradient of IL-8. IL-1 stimulated HUVEC cells will produce IL-8 at their cell surface where it presumably binds to heparin. By digesting heparin with our heparinase 3, the IL-8 will diffuse in media and neutrophils which follow the gradient will not be attracted to the cell surface, preventing them from migrating into the wells.

One filter on which HUVEC cells were growing was stained with crystal violet (see protocol on page 9). The cells were confluent. Media was taken out of the wells and IL-1 (2ng/ml) was added to 18 wells. The others received culture media only. After 4 hours the wells were emptied again, along with the inserts and Hep 3 at 1U/ml in PBS was added to both the wells and the inserts to 9 of them. The others received PBS only. The digestion was allowed to proceed for one hour at 37°C. One insert that received Hep 3 was then stained to see if the cells had detached and it appeared that some had in fact lifted. A different coating is going to be tried next time.

All the inserts were emptied along with the wells and 0.3ml of culture media was added to them. The wells and inserts that had some Hep 3 received culture media without heparin (RPMI + 20% FS). 1.5×10^6 neutrophils were added to every insert and then migration was

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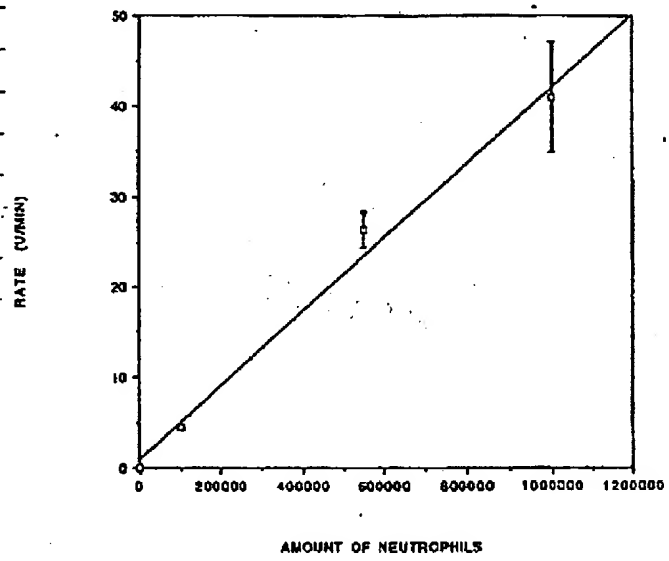
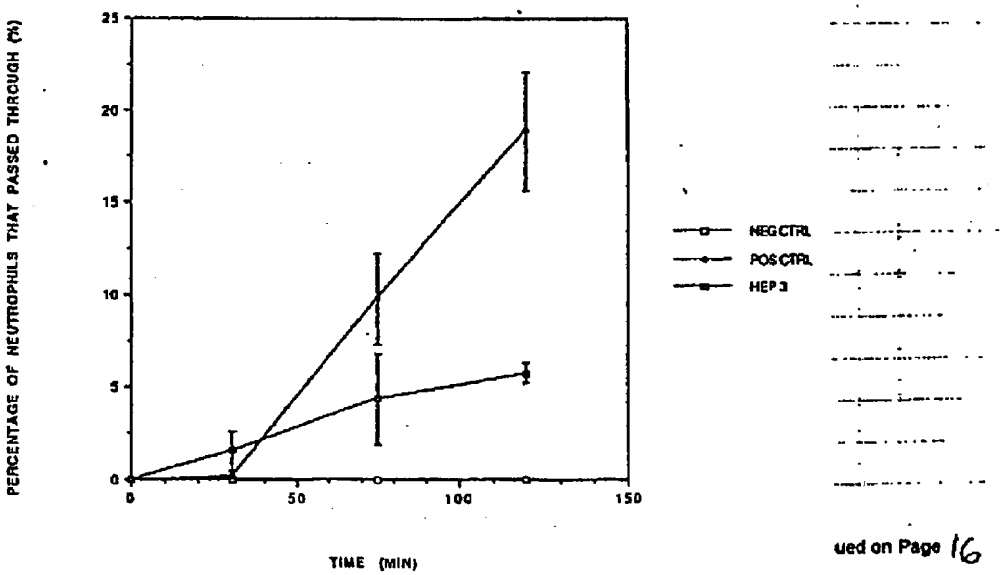
Stopped at 1 1/2 hr, 1 1/4 hr and 3 hours by taking out the inserts (3 filters of each series set time point). The bottom of the inserts were rinsed and the rinse was added to the content of the well. The samples were kept at -20°C O/n and amyeloperoxidase assay was done the day after.

MYELOPEROXIDASE ASSAY OF HUMAN NEUTROPHILS



MIGRATION OF NEUTROPHILS THROUGH A LAYER OF HUVEC CELLS DIGESTED WITH HEP 3 AT 1 IU/ML FOR AN HOUR



Raw Data
Re Raw
Data Binder
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